IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

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Application No.: 09/831,954 Group: 1617

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For: ESTROGENIC ESTRA-1,3,5(10)-TRIENES WITH DIFFERENTIAL EFFECTS ON THE ALPHA AND BETA ESTROGEN RECEPTORS, HAVING A LINEAR HYDROCARBON CHAIN FROM 5-9 CARBON ATOMS AT POSITION 11.

DECLARATION UNDER 37 C.F.R. §1.132

I, Antwan G. H. Ederveen declare as follows:

I am a pharmacologist, presently employed by N.V. Organon in the Netherlands as Section Head in the Pharmacology Department.

I was formerly a member of a lead optimization team, involved in pre-clinical research to find new estrogenic compounds for medical use.

I am familiar with the contents of the patent application for which this declaration is submitted to the US Patent Office and I am familiar also with the contents of Lobaccaro, C. et *al.*,'Steroidal Affinity Labels of the Estrogen Receptor 3. Estradiol 11-β-n-Alkyl Derivatives Bearing A Terminal

Electrophilic Group: Anti-Estrogenic and Cytotoxic Properties' *Journal of Medicinal Chemistry*, vol. 40, no. 14, July 4, 1997, pages 2217-2227 and Napolitano E. *et al.*, '11-β-Substituted Estradiol Derivatives 2. Potential Carbon-11- and Iodine Labeled Probes for the Estrogen Receptor' Journal of Medicinal Chemistry, vol. 38, no. 14, July 7, 1995, pages 2774-2779.

I declare that the information, which is provided in the following paragraphs of this declaration, is a truthful description of results of experiments performed in the laboratories of Organon and filed as such in our archives.

The binding affinity of compounds for the estrogen receptors can be determined in an *in vitro* assay with recombinant Chinese hamster ovary (CHO) cells stably transfected with the human estrogen receptor α (hERα) or β (hERβ). Competitive binding studies are performed using radiolabelled 17β-estradiol as a competitor ligand and the affinity of a test compound for the cytosolic estrogen receptors ERα and β is expressed as an IC_{50} ratio relative to 17β-estradiol (100%).

The agonist and antagonistic transactivation activity of compounds on the estrogen receptors can be determined in an *in*

vitro assay with recombinant Chinese hamster ovary (CHO) cells stably co-transfected with the human estrogen receptor α (hERα) or β (hERβ), the rat oxytocin promoter (RO) and the luciferase reporter gene (LUC). Agonist efficacy, *i.e.*, the amount of maximal activation of the receptor by a compound, is expressed as fractional activation and compared to the efficacy of 17β-estradiol (100%). A more detailed description of the methodology can be found in De Gooyer M.E., Deckers G.H., Schoonen W.G.E.J., Verheul H.A.M. and Kloosterboer H.J.,*Steroids,* Vol **68**, pp21-30 (2003).

The following results, obtained by these methods, are shown in a revised version of Table A, page 14 of the subject application:

Table A

A: Compound relative binding affinity at hERα.
B: Compound relative efficacy at hERα.
C: Compound relative binding affinity at hERβ.
D: Compound relative efficacy at hERβ.

Compound	A	B	Conclusion	C	D	Conclusion	Rating
1	136	1.32	agonist	87	1.58	agonist	-
2	99	1.19	agonist	82	1.17	agonist	-
3	116	0.51	agonist	96	0.00	antagonist	+
4	93	1.31	agonist	169	1.66	agonist	-
5	95	1.09	agonist	87	0.33	antagonist	+
6	155	0.78	agonist	126	0.00	antagonist	+
7	43	1.08	agonist	96	0.96	agonist	-
8	104	0.70	agonist	127	0.00	antagonist	+
9	170	1.35	agonist	150	1.60	agonist	-
10	190	1.19	agonist	115	0.57	agonist	-
11	70	0.46	agonist	71	0.00	antagonist	+

From the results it can be conciuded that all compounds (1-11) have good affinity for both estrogen receptor subtypes α and β as is evidenced by their relatively high binding affinity for hERα (Table A, column A)and hERβ (Table A, column C).

From the results it can also be concluded that substantially similar compounds can produce different functional effects at both estrogen receptor subtypes α and β. Thus whereas the 11β-butenyl derivative (compound 2) behaves as an agonist on ERα and an agonist on ERβ, the 11β-pentenyl homologue (compound 3) behaves as an antagonist on ERα but as an antagonist on ERβ.

Similarly, whereas the 11β-butynyl derivative (compound 4) behaves as an agonist on ERα and an agonist on ERβ, the 11β-pentynyl derivatives (compounds 5 and 6) behave as agonists on ERα but as antagonists on ERβ.

The differential properties observed between the C4 and C5 11β-homologues, as demonstrated in the table of results, is unexpected and does not follow in any way from the teachings of either Lobaccaro *et al.* or Napolitano *et al.*

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 17 U.S.C. §1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Number of pages of this declaration: 5 pages.

22-07-2004
Date

A. G. H. Ederveen